AD_________________

Award Number: W81XWH-07-1-0454

TITLE: Photonic Breast Tomography and Tumor Aggressiveness Assessment

PRINCIPAL INVESTIGATOR: S. K. Gayen, Ph. D.
J. A. Koutcher, M.D., Ph. D.
R. R. Alfano, Ph.D.
F. B. Lin, Ph.D.

CONTRACTING ORGANIZATION: University of New York City
New York, NY 10019-2925

REPORT DATE: July 2009

TYPE OF REPORT: Annual Summary

PREPARED FOR: U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

1. REPORT DATE *(DD-MM-YYYY)*
01-07-2009

2. REPORT TYPE
Annual Summary

3. DATES COVERED *(From - To)*
15 Jun 2008 – 14 Jun 2009

4. TITLE AND SUBTITLE
Photonic Breast Tomography and Tumor Aggressiveness Assessment

5a. CONTRACT NUMBER

5b. GRANT NUMBER
W81XWH-07-1-0454

5c. PROGRAM ELEMENT NUMBER

6. AUTHOR(S)
S. K. Gayen, J. A. Koutcher, X. Ni, M. Alrubaiee, Binlin Wu

E-mail: gayen@sci.ccny.cuny.edu

5d. PROJECT NUMBER

5e. TASK NUMBER

5f. WORK UNIT NUMBER

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)

University of New York City
New York, NY 10019-2925

8. PERFORMING ORGANIZATION REPORT NUMBER

9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)
US Army Medical Research and Materiel Command
Fort Detrick, MD 21702-5012

10. SPONSOR/MONITOR'S ACRONYM(S)

11. SPONSOR/MONITOR'S REPORT NUMBER(S)

12. DISTRIBUTION / AVAILABILITY STATEMENT

Approved for public release; Distribution unlimited

13. SUPPLEMENTARY NOTES

14. ABSTRACT
The tasks performed and the progresses made during the second year include: (a) pursuing planned training objectives of the CCNY researchers through laboratory rotations at the Memorial Sloan Kettering Cancer Center (MSKCC); and (b) conducting research on development of non-invasive optical imaging and spectroscopic approaches for breast tumor detection. The CCNY researchers received training on (i) small animal handling; (ii) Cell culture, bioluminescence assay and imaging; (iii) Western Blotting and Flow Cytometry; (iv) small animal imaging techniques; and (v) magnetic resonance imaging and spectroscopy. They also attended group meetings and seminars to develop sound background in the biological and clinical aspects of cancer research. The research component involved development of numerical algorithms based on time reversal matrix method and principal component analysis, and using those along with a method based on independent component analysis for locating tumors in a model cancerous breast assembled using *ex vivo* breast tissue specimens, and targets in breast simulating model media.

15. SUBJECT TERMS
Breast cancer, near-infrared imaging, optical tomography, independent component analysis, principal component analysis, small animal handling, bioluminescence, magnetic resonance imaging

16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT	18. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON
					USAMRMC
a. REPORT	**b. ABSTRACT**	**c. THIS PAGE**	Unclassified	15	**19b. TELEPHONE NUMBER** *(include area code)*
Unclassified	Unclassified	Unclassified			

Standard Form 298 (Rev. 8-98)
Prescribed by ANSI Std. Z39.18

Table of Contents

4. INTRODUCTION

The HBCU/MI Partnership Training Award project, "*Photonic Breast Tomography and Tumor Aggressiveness Assessment*," is designed to establish a breast cancer training and research program at the City College of New York (CCNY) through close collaboration with the researchers at the Memorial Sloan Kettering Cancer Center (MSKCC). The focus of the training component of the project is to familiarize the CCNY researchers who happen to be physical scientists and engineers to the biological aspects of cancer research through attending relevant courses, and cancer research practicum through laboratory rotations. The objectives of the research component of the project are to develop optical imaging and spectroscopic approaches to (a) distinguish between aggressive and slow growing, metastatic and non-metastatic tumors, (b) non-invasively detect and diagnose breast tumors at early stages of growth.

While the emphasis of the program is on the training component during the first two years, significant progress has been made in both training and research during the *second* reporting period (June 15, 2008 – June 14, 2009) covered by this report.

5. BODY

The tasks performed and the progresses made during the current reporting period are as follows:

- Accomplishment of planned training objectives through *laboratory rotations* and attending *group meetings and seminars* at MSKCC; and
- Pursuing research on development of non-invasive optical imaging and spectroscopic approaches for breast tumor detection.

We provide a brief outline of our accomplishments in these areas, and refer to appended materials for detailed description where applicable.

5.1. Accomplishment of Training Objectives

While the Year1 training involved accumulation of basic knowledge of the biological aspects of cancer research through course work, seminars, lectures, and workshops [1], the emphasis in the second year was on hands-on training through rotations in several breast cancer research laboratories at MSKCC (*Specific Aim 0, Task 2 and Task 3*), and keeping abreast of the recent developments in breast cancer research through participation in group meetings and seminars (*Specific Aim 0, Task 5*). The specialized training strategy included: training on animal handling at the Research Animal Resource Center (RARC) at MSKCC, and rotations through laboratories engaged in small animal imaging, and modern biomedical research methods. Specifics of the training received are described below.

5.1.1. Animal Handling Training

The trainees have attended the following small animal training sessions (*Specific Aim 0, Task 2*), held by RARC at MSKCC:

- *RARC Orientation* training session;
- *RARC Basic Mouse* training session that included handling and restraint; injectable anesthetics and anesthetic monitoring; parenteral administration (SC, IP, IV); blood sampling; and euthanasia;

- *RARC Rodent Survival Surgery* training session that involved surgical equipment and instruments, disinfection and sterilization, animal preparation, surgeon preparation, suture materials and wound closure, and post-operative care;
- *RARC Hazardous Material* training session that included introduction to the designated hazardous materials use areas, entry requirements, investigative staff responsibilities; animal/material manipulations, waste disposal/transportation of materials, and euthanasia.
- *RARC Xenograft* training session that focused on prevention of infectious disease transmission to laboratory researchers, animal care staff and other individuals who service the areas where these experiments are performed;
- *RARC special training on tail vein injection*; and
- *RARC special training on necropsy*.

5.1.2. Laboratory Rotations

The trainees rotated through the following four laboratories and received training on imaging techniques, cell culture, bioluminescence assay, Western Blotting, and other key techniques used in cancer research.

Rotation I

Laboratory PI: *Dr. Ronald Blasberg*

Laboratory background: The focus of the current research in this lab is on transgene (reporter gene) imaging using noninvasive nuclear and optical techniques.

Training Topic: *Cell culture and Bioluminescence Imaging*

- *Cell culture and bioluminescence training:* The trainees learned the basic cell culture techniques. Each of them had the responsibility to maintain two genetically modified breast cancer cell lines: MDA231-Fluc-I-GFP and MDA468-Fluc-I-GFP. Routine maintenance, such as subculture and cell counting, was performed.
- *Bioluminescence assay and imaging training:* The trainees learned the luciferase assay method for evaluating the level of expression of bioluminescence reporter gene (luciferin) and learned how to measure the linearity of bioluminescence intensity *vs* cell numbers using *Biospace* Photon Imager. One of the trainees identified saturation problems in the imaging system, which may cause misinterpretation of experimental results for large number of cells in plates or animals. He suggested a remedy that has been implemented to correct for the problem.

Rotation II

Laboratory PI: *Dr. David Solit*

Laboratory Background: This laboratory focuses on the development of cancer therapies that target pathways responsible for cancer initiation and progression.

Training Topics: *Western Blotting and Flow Cytometry*

The techniques that the trainees learned include:

- *Western blot* technique for detecting specific proteins related to RAS/RAF/MEK/ERk signal pathway: pERK, ERk, pMEK, MEK, Cyclin D1, p27, RB, PTEN, Cleaved PARP, and Caspase-3.
- *Drug (PD901) treatment and cell growth* curve measurement for OVCAR5, T24, and UMUC3 cell lines.
- *Flow cytometry technique* for cell cycle analysis. Nusse nuclear preparation method for DNA content was taught.

Rotation III

<u>Small-Animal Imaging Core</u>

<u>Facility Manager</u>: *Dr. Pat Zanzonico*

<u>Facility Background</u>: The imaging core facility provides MSKCC investigators with unique capabilities for noninvasively detecting, localizing, and biologically characterizing primary and metastatic cancer cells *in vivo* in small-animal models.

<u>Training Topics</u>: *Major small animal imaging techniques and systems*

The trainees learned how to use the following imaging equipment and techniques:

- Micro-PET PET (positron emission tomography)scanners from CTI-Concorde Microsystems ;
- Bioluminescence imaging system from Xenogen (Capiper) Corp.;
- Micro-CT CT (computed tomography) scanner from Imtek Corp.;
- SPECT / CT scanner from Gamma Medica;
- Ultra Sound from Visual Sonics; and
- Fluorescence imaging system from CRI.

Rotation IV

<u>Magnetic Resonance Imaging (MRI) Laboratory / Small-animal MRI Core</u>

<u>Laboratory PI</u>: *Dr. Jason Koutcher*

<u>Laboratory/Facility Background</u>: The MRI/MRS laboratory / facility are directed by Dr. Koutcher (Co-PI) and equipped with a 4.7 T and a 7 T magnet resonance imaging /spectroscopy systems.

<u>Training Topics</u>: *MRI / MRSI techniques*

The trainees participated in the following procedures and experiments:

- Design and build radiofrequency coils for MRI/MRS experiments;
- Acquisition and reconstruction of high resolution 1-H (standard) images in different orientations (axial, sagittal, and coronal) in small animals (up to cat size) and scans on mice whole-body, brain, lung, kidney, prostate, bladder cancers;

- Dynamical contrast imaging (DCE) technique for analysis of perfusion;
- Magnetic resonance spectroscopy of lactate for metabolite quantization of animals.

5.1.3. Other Training Activities

The CCNY and MSKCC group met regularly to discuss the progress during the Year 2 training. The trainees attend the weekly MSKCC MRI group meeting and selected seminars (*Specific Aim 0, Task 5*).

5.2. Development of Near-Infrared Optical Imaging Modality for Breast Cancer Detection

The research planned to be pursued in the project has two components: (a) development of non-invasive near-infrared optical imaging modalities for early detection of breast cancer, and (b) assessment of aggressiveness of tumor growth using an animal model. The research plan was organized such that work on developing the optical imaging modality would be undertaken early on, while the work on animal model would begin in the third year of the project.

Consequently, the work on development of non-invasive near-infrared optical imaging modalities for early detection of breast cancer (*Specific Aim 4*) that started during Year 1 of the project was further pursued throughout the current reporting period. This research builds on and extends the work that the CCNY group has been pursuing.

The goal of the research is to develop optical spectroscopy and imaging approaches that use the near-infrared (800-1300 nm) light to obtain three-dimensional (3-*D*) tomographic images of human breast that enable detection, localization, and possible diagnosis of tumor(s) in the breast. The work at the developmental stage would be carried out on phantoms that have optical absorption, emission, and scattering properties similar to those of breast tissues, and on realistic breast models assembled using *ex vivo* breast tissues. During this reporting period we further extended the scope and explored the efficacy of the *optical tomographic imaging using independent component analysis (OPTICA)* [2-5] that we developed earlier, and explored new approaches *optical imaging using principal component analysis (OIPCA)* and *time reversal optical tomography (TROT)*.

5.2.1. 'Model cancerous breast' studied using OPTICA

We extended OPTICA to include multi-wavelength probing and explored its efficacy on a more realistic model breast (*Specific Aim 4, Task #15 and Task #16*). The experimental arrangement for OPTICA, which we assembled (*Specific Aim 4, Task #14*) during the first reporting period [1], uses multi-source illumination of sample under investigation, and multi-detector transillumination signal acquisition. Light beams of wavelengths 750 nm, 800 nm, and 850 nm from a Ti-sapphire laser were used for probing. The 'model cancerous breast' was a 100 mm diameter and 42 mm thick cylinder assembled using of *ex vivo* adipose tissues ((*Specific Aim 4, Task #15*) with two tumors (invasive ductal carcinoma) embedded within. A 16-bit 1024 x 1024 pixels CCD camera was used to record the signal. The sample was scanned across the laser beam in a 16x26 *x-y* array of grid points and a two-dimensional transmission image was recorded for each position for each wavelength to meet the multi-source multi-detector (each pixel of a CCD camera being a detector element) imaging arrangement required for OPTICA. The data acquisition time is less than 8 minutes for a 16x26 scan at one given wavelength. The resulting data was analyzed using the numerical algorithm of OPTICA [2-3]. The details of the experimental arrangement, data acquisition, processing and analysis methods are similar to that presented in the First Annual Report [1] and a published paper [5].

The approach provided the locations of both the tumors in three dimensions with high accuracy. Multi-wavelength measurements enabled better discrimination of tumors from other components. A back-projection algorithm enabled estimation of the cross section of the tumors. The sample was further investigated using MRI and the results are in good agreement with the OPTICA estimates.

A paper entitled, "*Multi-wavelength optical tomography using independent component analysis*," based on these results has been accepted for presentation at the NIH Inter-Institute Workshop on Optical Diagnostic and Biophotonic Methods from Bench to Bedside 2009. A copy of the abstract that was submitted appears as *Appendix 1*. A manuscript is under preparation for publication in a professional journal.

5.2.2. Development of new optical imaging approaches

Fast and accurate methods are needed for detection and localization of tumours in breast, and for detection of margins during surgical removal of breast tumours. We have pursued the adaptation and development of two approaches *Optical Imaging using Principal Component Analysis (OIPCA)* and *Time Reversal Optical Tomography (TROT)* and tested those using experimental data on absorptive targets embedded in a breast tissue simulating model medium.

Principal component analysis (PCA) is a multivariate statistical method that captures the variance in a data set in terms of principal components and enables finding patterns in data of high dimension [6]. The components are assumed uncorrelated as opposed to the similar Independent Component Analysis (ICA) approach that assume the components to be independent. In our application involving detection of targets in a turbid medium, light propagation from different targets to the source plane and detector plane is considered to be uncorrelated and decomposed from the recorded data. The locations of the objects are retrieved by fitting the principal components of intensity distributions (PCID) to Green's functions.

The TROT approach [7, 8] is based on multi-static data and vector subspace classification to eigenvectors of a round-trip matrix. In optical imaging application, a *response matrix* represents the transport of light from multiple sources through a turbid medium with embedded targets to an array of detectors. The response matrix is constructed from the experimental data. The 'round-trip (RT) matrix' is constructed by multiplying the response matrix by its transpose matrix for continuous wave illumination (by adjoint matrix for frequency-domain case). Mathematically, the RT matrix is equivalent to transfer of light from multiple sources through a turbid medium with embedded targets to an array of detectors, and back, and is similar to the time-reversal matrix used in the general area of array processing for acoustic and radar time-reversal imaging [9].

Both OIPCA and TROT approaches use the same multi-source probing and multi-detector data acquisition scheme for obtaining multiple angular views of the target, as OPTICA does. To test the efficacy of these two approaches, we carried an experiment using Intralipid-10% suspension in water as a model medium, and two absorptive targets. The concentration of Intralipid-10% was adjusted to provide a transport mean free path $l_t \sim 1.43$ mm and an absorption coefficient $\mu_a = 0.003$ mm^{-1} at 785 nm. It was contained in a 250 mm × 250 mm × 50 mm transparent plastic container. The targets, two ~ 10-mm diameter glass spheres filled with indocyanine green dye solution in water ($\mu_a = 1.15$ mm^{-1}), were placed at 20 mm and 25 mm away from the entrance face, respectively. The sample was scanned at 11×12 grid points with a step size of 5 mm. The transmitted signal at 785 nm from the exit plane was recorded by a CCD camera. From each image, a 53.4 mm ×53.4 mm window was cropped out and then 5×5 pixels of each image were binned to one. Then OIPCA and TROT analysis were performed separately. A schematic diagram of the experimental arrangement, and information on data analysis and retrieval of target positions appear in *Appendix 2*.

Both OIPCA and TROT approaches were able to detect and obtain the positions of the two targets. The retrieved positions of the targets agreed well with known positions, and are compared in Table I.

Table I: Target positions determined by OIPCA and TROT

Objects	Known Positions (x, y, z) (mm)	OIPCA retrieved positions (x, y, z) (mm)	TROT retrieved positions (x, y, z) (mm)
1	(46.5, 14.5, 20)	(46.7, 14.7, 19.5)	(46.7, 14.7, 21)
2	(9.2, 44.5, 25)	(7.6, 44, 24.8)	(7.6, 43.2, 25)

Our future work will include exploring the efficacy of these approaches to detect and locate scattering targets, as well as, detecting tumours in realistic breast models, as was done for OPTICA (see Section 5.2).

6. KEY ACCOMPLISHMENTS

- The key training accomplishment includes successful introduction of physical scientists and engineers of CCNY research team to cancer biology research methods through laboratory rotations at selected MSKCC research laboratories.
- Key research accomplishments include: (a) demonstration of the efficacy of Optical Tomography using Independent Component Analysis (OPTICA) approach for detection, 3-*D* localization, and cross section imaging of two tumors inside a realistic breast model composed of excised breast tissues ; (b) exploration of the efficacy of approaches based two new algorithms for target detection and localization.

7. REPORTABLE OUTCOMES

Conference Presentations

(1) M. Alrubaiee, M. Xu, S. K, Gayen, V. Longo, and R. R. Alfano, "*Multi-wavelength optical tomography using independent component analysis*," accepted for presentation (Presentation Number: NIH09-NIH01-53) at the NIH Inter-Institute Workshop on Optical Diagnostic and Biophotonic Methods from Bench to Bedside 2009, October 1-2, Bethesda, MD.

(2) Binlin Wu, M. Alrubaiee, W. Cai, M. Xu and S. K. Gayen, "*Optical imaging of objects in turbid media using Principal Component Analysis and Time Reversal Matrix Methods,*" accepted for presentation at the Annual Meeting of the Optical Society of America, October 11-15, 2009, San Jose, California.

8. CONCLUSION

The work carried out during this reporting period: (a) continues the training of CCNY research team in biological and medical aspects of laboratory breast cancer research; and (b) shows the potential for noninvasive detection and three-dimensional localization of a tumor within a breast with significant accuracy. The contrast is based on the differences in the light scattering and absorption characteristics of the tumor and normal breast tissue.

"***So What Section***"

- The National Cancer Institute (NCI) has identified the development of imaging methodologies as an extraordinary opportunity for advancement in cancer research. Since the background of the CCNY team is in physical sciences and engineering, the training they received would provide them with necessary laboratory background in the biology of cancer research, and help develop a knowledgeable multidisciplinary research force in the fight against breast cancer.
- A recent study involving 35,319 patients underscores the influence of primary tumor location on breast cancer prognosis, and makes it imperative that breast cancer detection modalities obtain three dimensional (3-*D*) location of the tumor relative to the axilla [10]. The current work is an important development in obtaining 3-*D* location of a tumor within the breast.
- The study of model cancerous breast assembled using *ex vivo* breast tissues is important and essential for the next step, *in vivo* optical breast imaging involving volunteers.

9. REFERENCES

1. Annual Report Dated August 2008 covering the period 15 June 2007 – 14 June 2008, Award Number W81XWH-07-1-0454, on file at USAMRMC.
2. M. Xu, M. Alrubaiee, S. K. Gayen and R. R. Alfano, “Three-dimensional optical imaging of objects in a turbid medium using independent component analysis: theory and simulation,” *J. Biomed. Opt.* **10**, 051705 (2005).
3. M. Alrubaiee, M. Xu, S. K. Gayen, M. Brito, and R. R. Alfano, “Three-dimensional optical tomographic imaging of objects in tissue-simulating turbid medium using independent component analysis,” *Appl. Phys. Lett.* **87**, 191112 (2005).
4. M. Alrubaiee, M. Xu, S. K. Gayen, and R. R. Alfano, “Three-dimensional localization and cross section reconstruction of fluorescent targets in *ex vivo* breast tissue using independent component analysis,” *Appl. Phys. Lett.* **89**, 133902 (2006).
5. M. Xu, M. Alrubaiee, S. K. Gayen and R. R. Alfano, “Optical diffuse imaging of an *ex vivo* model cancerous human breast using independent component analysis,” *IEEE J. Select. Topics Quantum Electron.* **14**, 43 (2008).
6. M. Stetter, I. Schießl, T. Otto, F. Sengpiel, M. Hübener, T. Bonhoeffer and K. Obermayer, "Principal component analysis and blind separation of sources for optical imaging of intrinsic signals," *NeuroImage*, **11**, 482-490 (2000).
7. A. J. Devaney, "Time reversal imaging of obscured targets from multi-static data," *IEEE. Trans. Ant & Prop*. **53,** 1600-1610 (2005).
8. W. Cai, M. Alrubaiee, S. K. Gayen, M. Xu, R. R. Alfano, “Three-dimensional optical tomography of objects in turbid media using the round-trip matrix,” *Proc. SPIE* **5693**, 4 (2005).
9. C. Prada, J. L. Thomas, and M. Fink, “The iterative time-reversal process: analysis of the convergence,” *J. Acoust. Soc. Am.* **97**, 62 (1995).
10. N. Kroman, J. Wohlfahrt, H. T. Mouridsen, and M. Melbye, “Influence of tumor location on breast cancer prognosis,” *Int. J. Cancer* **105**, 542 -545 (2003).

10. APPENDICES

Appendix 1. A copy of the abstract of: M. Alrubaiee, M. Xu, S. K, Gayen, V. Longo, and R. R. Alfano, "*Multi-wavelength optical tomography using independent component analysis*," accepted for presentation at the NIH Inter-Institute Workshop on Optical Diagnostic and Biophotonic Methods from Bench to Bedside 2009, October 1-2, Bethesda, MD.

Appendix 2. A copy of the abstract of: Binlin Wu, M. Alrubaiee, W. Cai, M. Xu and S. K. Gayen, "*Optical imaging of objects in turbid media using Principal Component Analysis and Time Reversal Matrix Methods,*" accepted for presentation at the Annual Meeting of the Optical Society of America, October 11-15, 2009, San Jose, California.

Multi-wavelength optical tomography using independent component analysis

M. Alrubaiee[1], M. Xu[2], S. K, Gayen[1], V. Longo[3], and R. R. Alfano[1]

[1] Institute for Ultrafast Spectroscopy and Lasers, Physics Department
The City College of New York, 138th Street at Convent Avenue, New York, New York 10031
[2] Department of Physics, Fairfield University, 1073 North Benson Rd., CT 06824
[3] Zuckerman Research Center of Memorial Sloan-Kettering Cancer Center 1275 York Ave., NY, NY 10021

Multi-wavelength optical imaging using independent component analysis (MW-OPTICA) of a realistic breast model composed of *ex vivo* human female breast tissue is presented. The sample was a 100 mm diameter and 42 mm thick cylinder formed predominantly of adipose tissues with two tumors (invasive ductal carcinoma) embedded within. The experimental arrangement used 750 nm, 800 nm, and 850 nm light from a Ti-sapphire laser to illuminate the sample, and a 16-bit 1024 x 1024 pixels CCD camera to record the signal. The sample was scanned across the laser beam in a 16x26 *x-y* array of grid points and a two-dimensional transmission image was recorded for each position for each wavelength to meet the multi-source multi-detector imaging arrangement required for OPTICA. The data acquisition time is less than 8 minutes for a 16x26 scan at one given wavelength. The resulting data was analyzed using the ICA formalism.

The approach provided the locations of both the tumors in three dimensions with high accuracy. Multi-wavelength measurements enabled better discrimination of tumors from other components. A back-projection algorithm enabled estimation of the cross section of the tumors. The sample was further investigated using x-ray computed tomography (CT) for comparison with and testing the efficacy of optical approach. MW-OPTICA results are in good agreement with CT results.

The research is supported in part by US Army Medical Research and Materiel Command.

Optical Imaging of Objects in Turbid Media using Principal Component Analysis and Time Reversal Matrix Methods

Binlin Wu[1], M. Alrubaiee[1], W. Cai[1], M. Xu[2] and S. K. Gayen[1]
[1]*The Institute for Ultrafast Spectroscopy and Lasers, Physics Department*
The City College and the Graduate Centre of The City University of New York, New York, NY 10031, USA
[2]*Physics Department, Fairfield University, Fairfield, CT 06824, USA*
Email: bwu@sci.ccny.cuny.edu

Abstract: Principal Component Analysis and Time Reversal Matrix methods were used to develop approaches for imaging of targets in turbid media. The efficacy is demonstrated by imaging two targets embedded in Intralipid-10% suspension in water.

OCIS codes: (110.0113) Imaging through turbid media; (170.3880) Medical and biological imaging

1. Introduction

Fast and accurate methods are needed for detection and localization of targets embedded in turbid media. In this paper, we present two algorithms based on Principal Component Analysis (PCA) [1] and Multiple Signal Classification (MUSIC) to the eigenvectors of a Time Reversal (TR) matrix [2-3] for obtaining images and location information of targets in turbid media. We refer to these approaches as Optical Imaging using Principal Component Analysis (OIPCA) and Time Reversal Optical Tomography (TROT).

2. Formalism and Experimental Arrangement

A multi-source probing and multi-detector signal acquisition scheme to obtain multiple angular views of the target(s) was used for both methods. When using OIPCA, the leading eigenvalues provided the number and strengths of targets. Light propagation from different targets to the source plane and detector plane was considered to be uncorrelated and decomposed from the recorded data. The location of the objects was retrieved by fitting the principal components of intensity distributions (PCID) to Green's functions. When using TROT, a Time Reversal (TR) matrix was constructed by multiplying the response matrix by its adjoint matrix (transpose for continuous-wave (CW) illumination). The signal and noise subspaces were calculcated and separated using a method similar to L-curve regularization. The eigenvectors with leading non-zero eigenvalues of the TR matrix correspond to the embedded objects, which are orthogonal to the vectors in the noise subspace. The vector subspace method, MUSIC, along with Green's functions calculated from an appropriate forward model, was then used to determine the locations of the embedded objects.

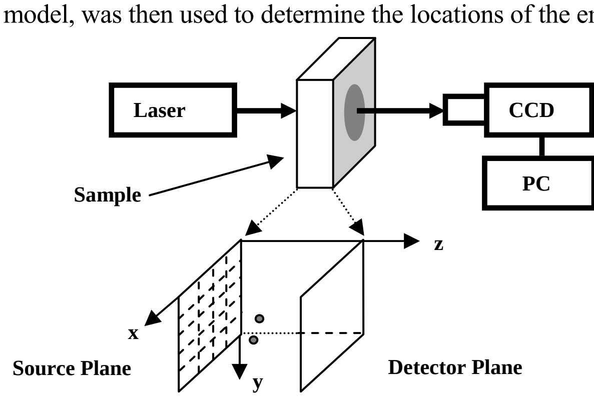

Fig. 1. A schematic diagram of the experimental arrangement used for imaging objects embedded in a turbid medium. Inset shows the 2D array in the input plane that was scanned across the incident laser beam.

The sample shown schematically in Fig. 1, was a 250 mm × 250 mm × 50 mm transparent plastic container filled with Intralipid-10% suspension in water, and two absorptive targets were embedded in it. The concentration of Intralipid-10% was adjusted to provide a transport mean free path $l_t \sim 1.43$ mm and an absorption coefficient $\mu_a = 0.003$ mm^{-1} at 785 nm. The targets, two ~ 10-mm diameter glass spheres filled with indocyanine green dye solution in water ($\mu_a = 1.15$ mm^{-1}), were placed at 20 mm and 25 mm away from the entrance face, respectively. The sample was scanned at 11×12 grid points with a step size of 5 mm. The transmitted signal at 785 nm from the exit plane was recorded by a CCD camera. From each image, a 53.4 mm ×53.4 mm window was cropped out and then 5×5 pixels of each image were binned to one. Then OIPCA and TROT analysis were perfermed separately.

3. Results

The OIPCA generated PCIDs of target 1 on the detector plane and source plane are shown in Figs. 2(a,c). Fitting of PCIDs to Green's functions are shown in Fig. 2(b,d). Similar images were obtained for target 2.

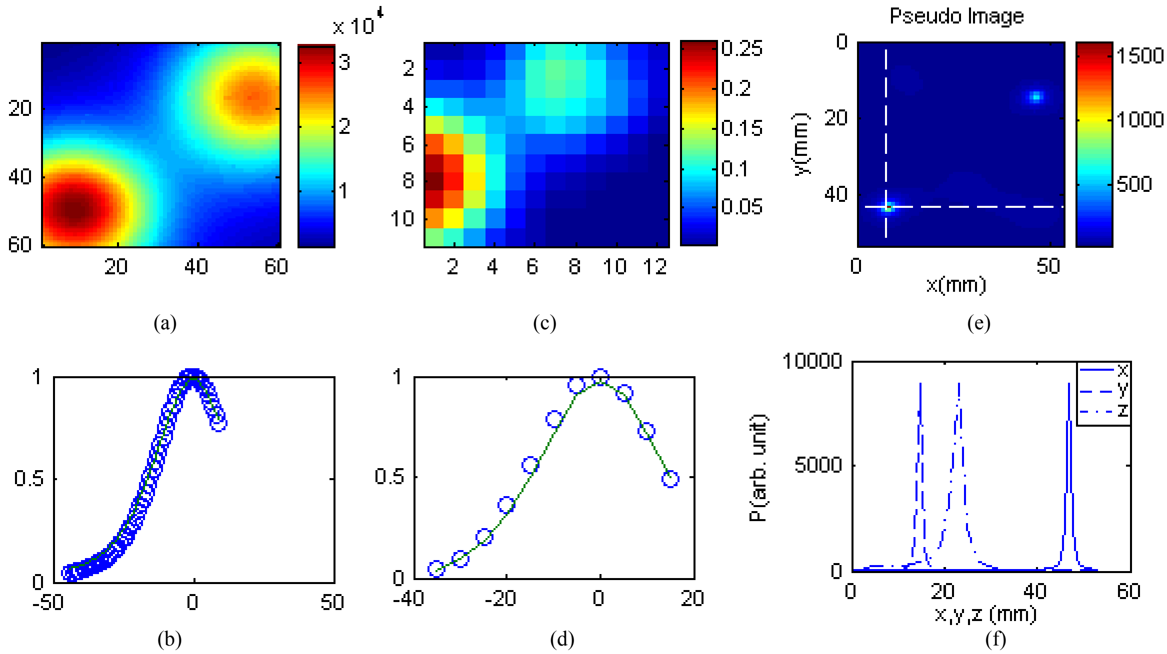

Fig 2. For target 1, OIPCA generated intensity distributions on the detector and source planes are shown in (a) and (c), respectively; Green's functions fit to the vertical spatial profiles through the maxima are shown in (b) and (d), respectively. TROT generated cross-section pseudo image is shown in (e) and pseudo value profiles through the target along x, y and z directions are shown in (f).

TROT found the absorptive objects and generated pseudo images using pseudo spectrum from MUSIC. The pseudo image and its profile for target 1 are shown in Fig. 2(e,f). Similar images for target 2 were obtained. Locations of the objects found by OIPCA and TROT are listed and compared with known locations in Table I.

Table I: Target positions determined by OIPCA and TROT

Objects	Known Positions (*x*, *y*, *z*) (mm)	OIPCA retrieved positions (*x*, *y*, *z*) (mm)	TROT retrieved positions (*x*, *y*, *z*) (mm)
1	(46.5, 14.5, 20)	(46.7, 14.7, 19.5)	(46.7, 14.7, 21)
2	(9.2, 44.5, 25)	(7.6, 44, 24.8)	(7.6, 43.2, 25)

4. Summary

Both methods detected the targets and provided their locations that are in good agreement with known positions. Experiments involving tumors in model breast assembled using *ex vivo* breast tissues are in progress.

The research is supported in part by US Army Medical Research and Materiel Command.

5. References

[1] M. Stetter, I. Schießl, T. Otto, F. Sengpiel, M. Hübener, T. Bonhoeffer and K. Obermayer, "Principal component analysis and blind separation of sources for optical imaging of intrinsic signals," *NeuroImage*, **11**, 482-490 (2000).

[2] A. J. Devaney, "Time reversal imaging of obscured targets from multistatic data," *IEEE. Trans Ant & Prop*. **53,** 1600-1610 (2005).

[3] W. Cai, M. Alrubaiee, S. K. Gayen, M. Xu, R. R. Alfano, "Three-dimensional optical tomography of objects in turbid media using the round-trip matrix," *Proc. SPIE* **5693**, 4 (2005).